

Occurrence of Arsenic in Plaice (*Pleuronectes platessa*), Nature of Organo-Arsenic Compound Present and Its Excretion by Man

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The arsenic content in 255 samples of plaice (*Pleuronectes platessa*) varied between 3 and 166 mg/kg. About 65% of the samples had an arsenic content above 10 mg/kg. High (low) arsenic concentration in the fillet corresponds with a high (low) concentration in milt or roe.

An excretion experiment with eight human volunteers showed that after the consumption of plaice, 69-85% of the ingested arsenic was excreted in the urine within five days. The organo-arsenic compound present in plaice was isolated by means of extraction, ion-exchange and thin-layer chromatography. Field desorption mass spectrometry of the isolate showed that arsenic was present as arsenobetaine, $(\text{CH}_3)_3\text{As}^+\text{CH}_2\text{COO}^-$.

Introduction

In general, high levels of arsenic are found in marine products. Chapman (1) observed high arsenic concentrations (2-132 mg/kg) among marine crustaceans and shell fish, whereas Schroeder and Balassa (2) reported arsenic levels ranging from 1.5 to 8.9 mg/kg in commercially available seafoods. High concentrations (2-38 mg/kg) in crabs were measured by Leblanc and Jackson (3), and concentrations varying between 0.15 and 18.7 mg/kg were found by Egaas and Braekkan (4) in 43 samples of fish products.

Westoö and Rydahl (5) determined the arsenic content of about 370 fish samples. Fish flesh, especially that from marine fish, and fish liver often showed arsenic levels above 1 mg/kg, the highest arsenic level being found in plaice (25 mg/kg). Various fish species have been analyzed for arsenic by Woidich and Pfannhauser (6). High concentrations were observed in conger eels (12-30 mg/kg), plaice (2-17 mg/kg), shrimp (0.4-12 mg/kg), sturgeon (0.7-12 mg/kg) and cod (2-10 mg/kg).

The World Health Organization (7) has published an acceptable daily intake of 50 μg arsenic/kg body weight. In view of the data given above, it appears that the consumption of marine products may be an important contribution to the total arsenic burden of human beings.

Chapman (1) showed that ~ 74% of arsenic (25 mg) present in lobster was excreted within 48 hr, whereas Coulson et al. (8) found that arsenic in shrimp was excreted completely within 4 days after consumption.

Schenk and Schreibeis (9) observed that the excretion of arsenic occurred mainly during the first 48 hr after consumption. Approximately 80% of the arsenic present in cod and plaice was excreted within 3 days (5). Munro (10), working with swine, observed that 68% of the arsenic from fish was excreted in the urine and 23% in the feces. Of arsenic from sole given to monkeys, 57-84% was excreted in the urine and 1-16% in the feces (11).

In 1977 Crecelius (12) reported the concentration of four different arsenic compounds [As^{3+} , As^{5+} , $\text{CH}_3\text{AsO}(\text{OH})_2$ and $(\text{CH}_3)_2\text{AsO.OH}$] in urine after the ingestion of arsenic-rich substances (wine, well water, crabmeat). Arsenic present in wine as As^{3+} and As^{5+} was excreted 50% as dimethylarsinic acid, $(\text{CH}_3)_2\text{AsO.OH}$, 8% as As^{3+} , 8% as As^{5+} and 14% as

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methylarsonic acid [$\text{CH}_3\text{AsO}(\text{OH})_2$]. It has been shown by Lakso and Peoples (13) and Tam et al. (14) that ingested inorganic arsenic will be metabolized as methylated arsenic. After the ingestion of crabmeat containing an unidentified organo-arsenic compound, the urinary concentration of the four arsenic compounds did not change significantly. However, when urine samples were digested with hot 2N NaOH, high concentrations of dimethylarsinic acid were present.

The behavior and toxicological properties of arsenic depends on the chemical form in which it is present. Penrose (15) mentioned that the toxicity decreases in the following way; arsines (trivalent inorganic or organic) > inorganic arsenite > arsenoxides > inorganic arsenate > pentavalent arsenicals > arsonium compounds (four organic groups with a positive charge on arsenic) > metallic arsenic.

Several investigators have made more or less successful attempts to elucidate the structure of the organo-arsenic compound(s) in marine fish. Lunde (16) and Brooke and Evans (17) reported the separation of inorganic arsenic (As^{3+} and As^{5+}) from organic bound arsenic in fish by distillation from hydrochloric acid. Although the distillation conditions were rather drastic, only small amounts of inorganic arsenic (0.01-2.5 mg/kg) were present.

Edmonds and Francesconi (18) showed that digestion of methanol extracts of mussels, western rock lobster and sting ray in aqueous sodium hydroxide led, after neutralization, to a solution from which the arsines, $(\text{CH}_3)_2\text{AsH}$ and $(\text{CH}_3)_3\text{As}$, were generated after reduction with sodium borohydride. The organo-arsenic compound isolated from western rock lobster by Edmonds et al. (19) has been identified as arsenobetaine, $(\text{CH}_3)_3\text{As}^+\text{CH}_2\text{COO}^-$.

Penrose et al. (20) isolated an organo-arsenic compound from witch flounder which possessed a mass spectrum that resembles tetramethylarsonium. The anion-exchange and thin-layer properties of the isolated compound, however, did not correspond with those of $(\text{CH}_3)_4\text{As}^+$. The structure of the isolated organo-arsenic compound from witch flounder has not been further elucidated.

Cannon et al. (21) reported the presence of arsenobetaine in dusky shark (*Carcharhinus obscurus*).

Kurosawa et al. (22) used HPLC and field desorption mass spectrometry for the identification of arsenobetaine in the muscle and liver of a shark (*Prionace glaucus*).

Recently, Edmonds and Francesconi (23) showed that the thin-layer properties of organo-arsenic isolated from school whiting are identical to those of arsenobetaine. It was therefore concluded that arsenic in school whiting is present as arsenobetaine.

Benson and Summons (24) mentioned that arsenic isolated from kidney tissue of a giant clam is mainly present as trimethylarsonium lactate and O-glycero-phosphoryl trimethylarsonium lactate.

Still more information about arsenic in marine products is necessary. This paper describes an investigation into the occurrence of arsenic in an important flatfish species, plaice (*Pleuronectes platessa*), and gives some results of studies on the nature of an organo-arsenic compound present therein and the excretion by man.

Material and Methods

Sampling

During the period February 1979-March 1980, 255 samples of plaice (*Pleuronectes platessa*), caught at different fishing grounds in the North Sea, were obtained from the Netherlands Institute for Fishery Investigations, Ijmuiden, the Netherlands. The edible parts, liver, milt and roe, were homogenized separately and stored at -20°C until the analysis. Table 1 presents a survey of the sampling data.

Determination of Arsenic

Total Arsenic. The homogenized samples were dry-ashed overnight at 550°C in the presence of $\text{Mg}(\text{NO}_3)_2$ and MgO . After dissolving the ash in hydrochloric acid, As^{5+} was reduced to As^{3+} with the addition of potassium iodide.

Arsine, formed by the use of zinc-hydrochloric acid as hydride generator, was absorbed in a solution of silver diethyldithiocarbamate in pyridine. The absorbance at 528 nm was then measured.

Methylated Arsenic. A homogenized fish sample was submitted to a Bligh and Dyer extraction (25). After evaporation of methanol from the aqueous/methanol phase at 40°C , the aqueous fraction was digested in hot 4N KOH for 2 hr. In the case of urine samples, the Bligh/Dyer extraction procedure was omitted. After the KOH digestion the sample could be analyzed for inorganic and methylated arsenic by reduction with sodium borohydride at $\text{pH} < 1$. During the reduction procedure the hydrogen and the arsines formed were collected in a 2-liter bottle filled with deionized water which was placed upside down in a water bath. The collection was terminated after 2 min and the bottle was then closed under water with a septum. In preliminary experiments it was established that under these conditions the arsenic present was recovered completely. A 1 ml gas sample was submitted to gas chromatography. Detection was performed with a

Table 1. Concentration of arsenic in plaice (*Pleuronectes platessa*).

Date	Number of samples	Arsenic concentration, mg/kg		
		Average	Range	Median
790215	10	48	9-166	24
790301	10	29	18-49	27
790504	21	15	4-70	11
790522	20	16	8-35	14
780615	20	11	3-19	13
790713	20	57	15-119	60
790907	20	14	4-40	12
790927	20	11	6-21	11
791101	16	15	5-31	12
791129	20	11	3-53	7
791221	18	19	5-77	10
800117	20	28	3-97	19
800222	20	24	8-86	15
800327	20	41	6-144	34
Total sampling period	255	23	3-166	14

microwave-induced plasma detector. The conditions used for the separation of AsH_3 , CH_3AsH_2 , $(\text{CH}_3)_2\text{AsH}$ and $(\text{CH}_3)_3\text{As}$ were as follows. Glass column, $1.8 \text{ m} \times 1.8 \text{ mm}$ ID packed with 5% Carbowax 20M on Chromosorb 101 (80-100 mesh); column temperature, 170°C ; injection port temperature, 200°C ; wavelength, 235.0 nm; split width, 0.2 nm; microwave generator output, 75 W; carrier gas, He; capillary pressure, 50 mm Hg. Solutions of As_2O_3 , $\text{CH}_3\text{AsO}(\text{ONa})_2$, $(\text{CH}_3)_2\text{AsO}.\text{ONa}$ and $(\text{CH}_3)_3\text{AsCl}_2$ were used as standards.

Isolation Procedure for Organo-Arsenic Found in Plaice

The isolation procedure for the organo-arsenic compound found in plaice consisted of three steps; extraction, ion-exchange and thin-layer chromatography.

About 210 of plaice (166 mg As/kg) was subjected to a lipid extraction according to the Bligh and Dyer procedure (25). Methanol was evaporated from the methanol/aqueous phase at 40°C . The aqueous extract, acidified by adding HCl until a pH 3 was reached, was then passed through a cation-exchange column (Amberlite IR-120, 20-50 mesh, H^+ form). Elution of the retained organo-arsenic compound was performed with 2% NH_4OH . After neutralization, the eluate was applied to a mixed-bed column (Amberlite, IRA-400, 20-50 mesh, OH^- form, Amberlite IRC-50, H^+ form). The eluate, which contained the organo-arsenic compound, was subjected once again to the cation-exchange procedure. Fractions of the NH_4OH elution step containing the arsenic-compound were then collected. The overall recovery of arsenic from plaice by the described isolation procedure was about 80%.

Thin-layer chromatography of the isolate on cellulose with *n*-butanol/acetic acid/water (60:15:25) resulted in three spots ($R_f = 0.65, 0.58$ and 0.48) after exposure to iodine vapor. Only the spot with $R_f = 0.58$ contained arsenic. This arsenic-containing spot was then extracted with methanol and subjected to field desorption mass spectrometry.

Excretion Experiment

Eight healthy human volunteers (6 male, 2 female) each consumed 100 g plaice (80 mg As/kg). Prior to this experiment none of the participants had eaten arsenic-rich food. During the first 12 hr of the experiment the volunteers were asked to urinate at 2 hr time intervals. After 5 days the experiment was terminated. All urine samples were analyzed for the presence of arsenic according to the procedure described above for methylated arsenic.

Results and Discussion

The average and median concentrations and the concentration ranges of arsenic in filets of plaice are given in Table 1. A frequency distribution of arsenic over the 255 investigated samples of plaice is given in Figure 1. A large variation in the arsenic content was observed. About 65% of the samples had an arsenic content above 10 mg/kg.

The arsenic content in the filet, liver, milt and roe of 18 samples of plaice (Table 2) show that a high (low) arsenic content in the filet corresponds to a high (low) arsenic level in the liver and milt. In some liver samples the arsenic content was several times higher than in the corresponding filets. It is not clear to what extent this was due to accumula-

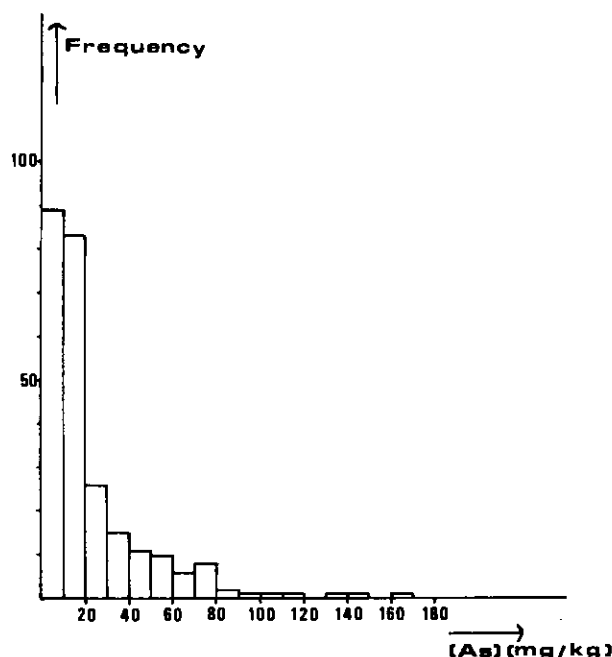


FIGURE 1. Frequency distribution of arsenic in 255 samples of plaice.

tion of arsenic in the liver. Compared with the fillets, the arsenic content in roe is low.

The excretion of arsenic in the urine of eight human volunteers after 5 days varied between 69 and 85% (average 75%) of the administered dose. As an illustration the arsenic content in the urine of two volunteers is given as a function of the time in Figure 2. The bulk of the ingested arsenic was

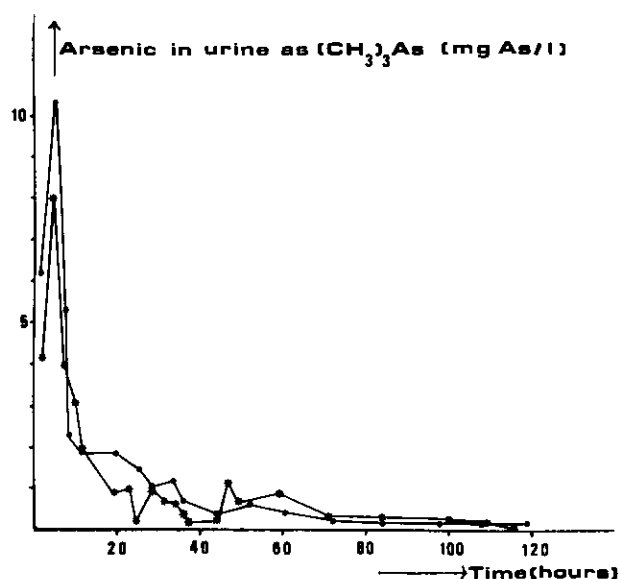


FIGURE 2. Concentration of arsenic in urine, as $(\text{CH}_3)_3\text{As}$, as a function of time after the ingestion of plaice by two human volunteers. The urine samples were analyzed for the presence of arsenic following the procedure described in the section on methylated arsenic.

excreted during the first 24 hr after consumption.

Only small amounts of inorganic arsenic (< 30 ng/ml) were present in the urine samples prior to the excretion experiment. All the arsenic excreted after consumption of plaice was present as trimethylarsine in urine after alkaline digestion and reduction with NaBH_4 , at low pH. Other forms of methylated arsenic as well as inorganic arsenic

Table 2. Arsenic content in fillet, liver, milt or roe of 18 samples of plaice.

Sample	Sex	Arsenic concentration, mg/kg			
		Fillet	Liver	Milt	Roe
1	F	135	318	—	6.5
2	F	29	26	—	11
3	F	60	53	—	17
4	F	47	92	—	1.3
5	F	97	89	—	4.9
6	F	86	431	—	12.4
7	F	34	126	—	3.8
8	M	77	44	42	—
9	M	76	199	65	—
10	M	57	75	43	—
11	M	103	83	28	—
12	M	73	44	72	—
13	M	5.3	2.4	2.6	—
14	M	5.4	1.3	2.3	—
15	M	5.8	2.3	1.9	—
16	M	6.1	2.8	2.5	—
17	M	5.8	1.5	2.8	—
18	M	5.9	3.6	2.7	—

Table 3. Some properties of organo-arsenic compounds.

Compound	After the alkaline digestion/reduction procedure	R_f (BuOH/HAC/H ₂ O)
Arsenobetaine, $(\text{CH}_3)_3\text{As}^+\text{CH}_2\text{COO}^-$	$(\text{CH}_3)_3\text{As}$	0.58
Arsenocholine, $(\text{CH}_3)_3\text{As}^+\text{CH}_2\text{CH}_2\text{OH}$	Trace $(\text{CH}_3)_3\text{As}$	0.58
Tetramethylarsonium iodide, $(\text{CH}_3)_4\text{As}^+\text{I}^-$	—	0.35
Organo-arsenic from plaice	$(\text{CH}_3)_3\text{As}$	0.58

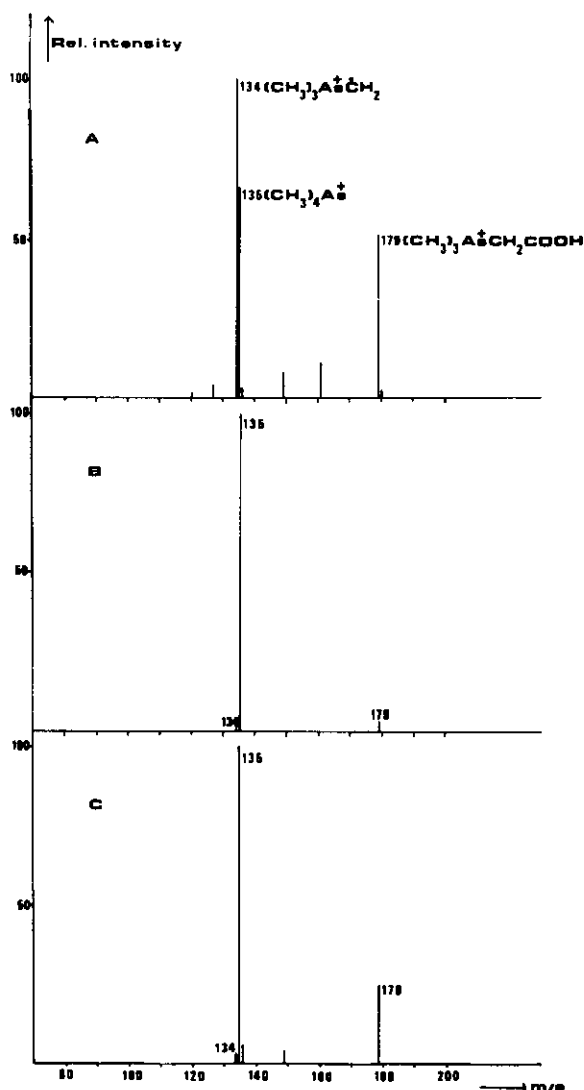


FIGURE 3. Field desorption mass spectra of (A) arsenobetaine, (B) the organo-arsenic compound from plaice and (C) a mixture of the two.

were not present. It was established that no volatile arsines were present if the alkaline digestion is omitted prior the reduction procedure. The necessity of an alkaline digestion prior to the reduction procedure agrees with the results of Crecelius (12). However, in this investigation trimethylarsine is formed in urine after reduction, while Crecelius reported the formation of dimethylarsine.

It was demonstrated that the bulk of the arsenic in plaice (95%) was present in the aqueous fraction after the Bligh and Dyer extraction. Digestion with KOH followed by a reduction with NaBH_4 led to the formation of $(\text{CH}_3)_3\text{As}$. The properties of the organo-arsenic reference compounds after the digestion procedure as well as the R_f values, given in Table 3, indicate that the isolated organo-arsenic compound found in plaice was neither arsenocholine nor tetramethylarsonium.

The mass spectra obtained by field desorption mass spectrometry of the isolated organo-arsenic compound, arsenobetaine and a mixture of the two shown in Figure 3. According to the molecular structure of arsenobetaine the protonated molecular-ion $(\text{CH}_3)_3(\text{As}^+\text{CH}_2\text{COOH})$ ($m/e = 179$) and two fragments $(\text{CH}_3)_4\text{As}^+$ ($m/e = 135$) and $(\text{CH}_3)_3\text{As}^+ + \text{CH}_2$ ($m/e = 134$) are present. The peak at $m/e = 134$ was the base peak. The mass spectrum of the isolated organo-arsenic compound showed the presence of an ion at $m/e = 135$ as the base peak and an ion at $m/e = 179$ with small intensity. No peak at $m/e = 134$ was present. The addition of an equivalent amount of arsenobetaine to the isolated compound caused a significant shift in the fragmentation pattern of pure arsenobetaine. The nominal mass $m/e = 135$ became the base peak, while the relative intensities of $m/e = 134$ and $m/e = 179$ decreased considerably. Protonization of arsenobetaine, probably due to impurities present in the isolated compound, increased the presence of the tetramethylarsonium ion.

High resolution field desorption mass spectrometry ($R = 10^4$, 10% valley definition) showed that the exact mass of $m/e = 179$ of arsenobetaine and that of the isolated organo-arsenic compound did not

differ significantly from each other. It was therefore concluded that the arsenic found in plaice is present as arsenobetaine.

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